The Occurrence of Situs inversus among artificially-reared Echinoid Larvae.

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With 3 Text-figures.

Contents.

1. Introduction.

A remarkable case, where a hydrocoele and its associated structure had developed only on the right side instead of on the left side of the body, as in normal specimens, came under my notice among the artificially reared larvae of Echinus miliaris.

Cases of situs inversus viscerum are not very rare in nature, and are frequently met with under artificial conditions. Spemann (**29**, pp. 400–14), in his most interesting experimental studies on Triton larvae, has made an exhaustive survey on cases of situs inversus. According to him (p. 401) the cases may be classified into two categories, though the distinction between these two may not be clean-cut. The one comprises such cases where an 'inverting' factor affects an individual very early in its ontogeny, it may be even before fertilization, so that the 'microstructure' of the egg undergoes a change at once and completely. Those Gasteropods with reversed spiral belong to this category. Conklin (**3**, p. 585) suggested as its cause the reversal of the polarity in the egg.

To the second belong those cases where that factor acts much later in the embryonic development, a little while previous to the time when any visible asymmetry of organization occurs. It affects only a single but decisive part, and in consequence of the abnormal development of that part all the other adjoining organs will assume the inverse situs. There are many interesting instances of this: thus, for example, a chick embryo heated on its left side (Dareste, Warynski and Fol), a Triton embryo with a portion of the medullary plate cut out and replaced in the inverted position (Spemann), an egg or embryo which has been constricted along its median plane partially or completely so as to give rise to either a double monster or twins (Spemann; compare Bateson, **2**, p. 560, and Morrill, **18**, p. 267), and two halves of embryos with different rate of growth grafted together (Spemann) can likewise produce the situs inversus. Cases of such partial situs inversus have also been interpreted in a most satisfactory manner, as has also the striking fact that generally the abnormality is exhibited by the right-hand members of double monsters of Triton (and trout) and by the right-hand member of twin Triton larvae.

Turning now to the case of the reversed Echinus larvae, I have tried to propose tentatively an interpretation. This case is, it seems to me, more or less related to, but distinct in some respects from, the above-mentioned second category (see p. 141). The idea came to my mind after the experiments had come to an end, and it needs further test with special reference to this question.

The experiments were made during the early summer of this year (1920) in the Zoological Department, Imperial College of Science and Technology, London. It is my pleasant duty to tender my hearty thanks to Professor E. W. MacBride for his kind supervision and unceasing encouragement throughout the time during which the work has been carried out. The writing of the manuscript was done in the Natural History Department of the British Museum. My cordial gratitude is also due to Sir Sidney F. Harmer, Director of the Department, for his kind permission to work there and to use the library.

2. Descriptions of the Larvae with Inverse Situs.

It must at the outset be stated with regret that the descriptions of internal structures as here given are founded on a very few specimens which I could preserve and section. As will be seen in the table (p. 115) the total number of reversed larvae I found was more than 150, but with the hope of getting as many metamorphosed young as possible I did not kill and preserve many of them. The observation on the early stage when the right hydrocoele makes its appearance, i.e. the earliest visible sign of the abnormality, is also lacking. About half a dozen metamorphosed young were obtained, but all the rest died off gradually without affording me any opportunity of following the internal changes which had taken place.

External Characters.—Eight larvae with the inverse situs were first found on May 31, when they were eleven days old. The 'larval' body was quite normal both in size and shape: two pairs of larval arms, post-oral and antero-lateral, both symmetrical and fairly long; postero-dorsal arms still very short; the posterior part of the body-rod beginning to degenerate, with its club-shaped end separated from the rest and lying near the hind end of the body. Both the ventral and dorsal epaulettes were already separated from the ciliary bands, the anterior transverse part of the latter showing a peculiar twist which indicated the future position of the paired pre-oral arms.

A hydrocoele, stone-canal, and amniotic invagination were all situated on the right side, whilst no such organs were found on the left side. No special attention was paid to such a slight asymmetrical distortion in shape of the stomach as was often noticed by Runnström in some abnormal larvae (**24, 25**).

Similar larvae were found later to be fairly numerous, and were transferred to a separate jar where they were allowed to develop further. There was found no difference in the rate of growth between normal larvae and these abnormal ones. When fully grown (Text-fig. 1) the abnormal larva possessed four pairs of well-developed arms, a large echinus-rudiment (*rd*) on the right side, from which five primary tentacles often protruded and moved actively. Whether a pair of pedicellariae really appeared on the left side as is the case with Strongylocentrotus (see p. 136) I cannot assert at present, though it seems to me to be highly probable. As to those paired calcareous structures which appeared on the left side, as seen in the text-figure (sp_2), I am almost certain that they were groups of spines.[1] The unpaired spine which should appear in normal cases at the hind end, a little to the right of the median line, was here found shifted to the left side (sp_1).

No less than half a dozen of such abnormal larvae passed metamorphosis when a month old. As to the external feature of these young sea-urchins one can find no difference from

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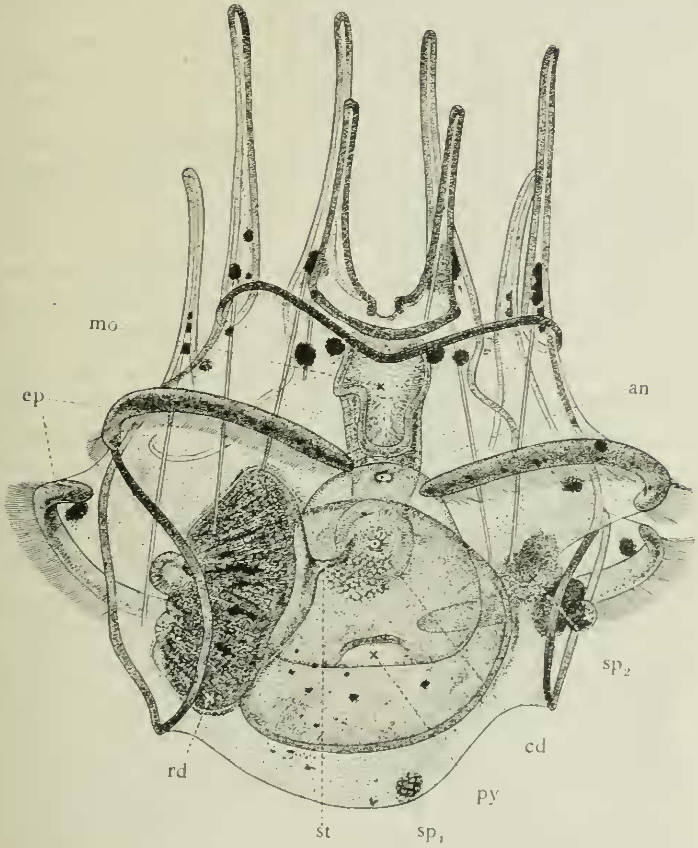
[1] While dealing with the living larvae I thought without the slightest doubt that the paired calcareous structures always found on the left side were really pedicellariae. Text-fig. 1, which is the only drawing made of this stage from life and the only evidence now available, shows that they are situated inside the loop of the ciliary band. This position coincides precisely with that of the groups of spines as described by Runnström (**27**, pp. 21–2, figs. 21–3). In this particular specimen at least there were present no true pedicellariae (see p. 138).

TEXT-FIG. 1.



Full-grown larva of Echinus miliaris with inverse situs. Ventral view. ×75.

an, anus; *cd*, constriction between larval oesophagus and stomach; *ep*, ventral and dorsal epaulettes; *mo*, larval mouth; *py*, constriction between larval stomach and intestine; *rd*, echinus-rudiment formed on the right side; sp_1, rudiment of posterior unpaired spine situated a little on the left to the median line; sp_2, a pair of groups of spines formed on the left side.

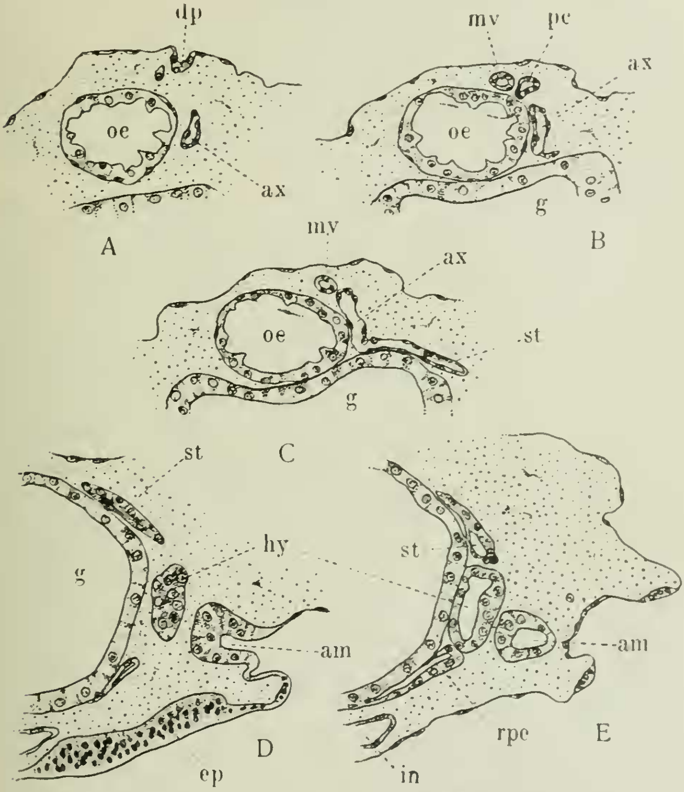
normal young. All the sets of primary unpaired and first-paired tentacles, pedicellariae, pointed and square-ended spines, were formed precisely as in the young which had metamorphosed from normal larvae. It was hoped that they would develop further to the stage when the asymmetrical arrangement of the organs, above all the peculiar coil of the intestine, would be more pronounced. Unfortunately, however, they were all lost after ten days, probably being destroyed by a tiny Gasteropod which had been carelessly put into the jar together with some Corallinae.

Internal Structures.—So far as the internal anatomy of the larva is concerned, the following short account is all we can learn. The transverse sections of the larva (Text-fig. 2) are exactly the mirror-images of those of normal larvae, so that one cannot distinguish them from sections of a normal larva mounted upside down. An eleven-day-old larva has the pore-canal (*pc*) still distinctly opening on the right side of the mid-dorsal line (*dp*), a madreporic vesicle (*mv*) lying close to the canal, situated at its median side but without any communication whatever with it. The canal then leads to the thin-walled axial sinus (*ax*) which lies close to the oesophagus (*oe*). The stone-canal (*st*) connects the axial sinus and the hydrocoele just as in the normal case. The hydrocoele (*hy*) situated on the right side of the stomach (*g*) has just begun to produce lobes, and an amniotic invagination (*am*) has already appeared. No traces of hydrocoele, stone- and pore-canals were found on the left side. From want of material it is not known on which side of the posterior coelom the genital stolon would be formed.

Thus, with doubtful exception of the pedicellariae and genital stolon, the internal organs as well as the external characters showed perfectly the inverse situs in every detail, so far as I could examine. With regard to the pedicellariae and genital stolon I refrain from expressing a definite opinion. We may expect to find some aberrant types as might be suggested from further descriptions of double-hydrocoele larvae.

Similar cases previously known.—So far as I am aware, the similar cases among Echinoid larvae have only been recorded twice by Runnström. He described two such larvae of Strongylocentrotus lividus reared at Monaco.

TEXT-FIG. 2.



Transverse sections of an eleven-day-old reversed larva of Echinus miliaris. ×300.
am, amniotic invagination ; *ax*, axial sinus ; *dp*, dorsal pore ; *ep*, ciliary epaulette ; *g*, stomach ; *hy*, hydrocoele ; *in*, intestine ; *mv*, madreporic vesicle ; *oe*, oesophagus ; *pc*, pore-canal ; *rpc*, right posterior coelom ; *st*, stone-canal.

[Case A.] Runnström, 1912 (**23**), pp. 2–3, ‘ no. 1 ’ ; 1918 (**26**), pp. 419–20.

Left : no hydrocoele developed, but instead of it a ventral primary pedicellaria was formed.

Right: echinus-rudiment well developed. Dorsal pore remaining at its original position on the right of the mid-dorsal line, no shifting towards the latter taking place.

[Case B.] Runnström, 1912 (**23**), pp. 7–10, 'no. 5'; 1918 (**26**), pp. 420–4, Taf. xiv, figs. 12–16.

Left: hydrocoele not formed, anterior coelom remaining rudimentary. Amniotic invagination formed as only a shallow depression, and afterwards disappearing. Pedicellariae present, the dorsal one being already formed while the ventral one was indicated by accumulated cells.

Right: anterior coelom consisted of two portions, one being the axial sinus communicating with stone-canal and the other representing the madreporic vesicle (' pulsating organ '), which displayed infrequent and irregular pulsation. The dorsal pore was not at first formed, though an ectodermal groove indicated it. After some days a pore opened anew, and the madreporic vesicle began to pulsate more frequently and regularly than before. The stone-canal became split into two parts, one short and still communicating with the hydrocoele whilst the other was longer and opened freely into the coelom.[1] These two then degenerated and a new stone-canal appeared, so that the hydrocoele regained its communication with the exterior. The hydrocoele produced two diverticula, one of the ordinary size and the other much larger. The amniotic invagination was not formed, but instead of it there were two ectodermal pits. These the author at first (**23**) interpreted as rudiments of the primary pedicellariae, but afterwards (**26**) corrected his former view and called them ' spine invaginations ' (see p. 138).

In other classes of Echinoderms, Auriculariae with the hydrocoele on the right side only were noticed by Müller many years ago (**19**, pp. 101, 109, Taf. v, fig. 1). From communications with Dr. Th. Mortensen I have learnt that he found among the larvae of Ophionotus hexactis two specimens which had a right hydrocoele only. I here

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[1] ' Cavité générale ' and ' Körperhöhle ' in the original descriptions. Under these terms the posterior coelom is probably meant.

express my thanks to Dr. Mortensen for the kind permission to note this discovery, which he has not yet published.

3. Results of the Experiments.

The purpose of our experiments made under the direction of Professor MacBride was to carry out a further test of the influence of high salinity on the production of double hydrocoeles (**15,** pp. 334–7, 341). Fresh specimens of Echinus miliaris were sent from Plymouth, ripe males and females were then selected from among them, and the eggs were fertilized. For detailed descriptions of the method we adopted I refer to MacBride's paper (**15,** pp. 326–9). Only a few details need be added here (see table on p. 115). 'Outside water' of Plymouth (**1,** p. 372) was always used in starting the culture, viz. the eggs were fertilized in it and then kept for a day in finger-bowls filled with clean 'outside water' ('finger-bowl period'). One-day-old larvae with pyramidal body and a pair of rudimentary post-oral arms were then transferred to Breffit jars, which had been filled with 'outside water' supplied with some Nitzschia ('Breffit-jar period'). Then some of them were treated for several days with 'hypertonic' sea-water, which had been synthetically prepared according to Allen and Nelson (**1,** pp. 369–71), and the salinity increased roughly to 3·7 per cent. while others were left untreated as controls. When about a fortnight old, the larvae were put into plunger jars, which had been filled with synthetic sea-water of normal salinity mixed with a small quantity of 'outside water' ('plunger-jar period'). The results of five more or less successful cultures are here shown in the table. They were offsprings of three different parents: culture nos. 1 and 4 belonging to the first, nos. 6 and 9 to the second, and no. 11 to the third. The larvae with the inverse situs were first discovered among no. 4, on May 31. Those fifty-four abnormal larvae of this culture were then kept separate in a Breffit jar. On June 19, thirty days after fertilization, some few among the normal ones of this culture were found just metamorphosed into tiny young sea-urchins, while one of those fifty-four abnormal larvae also metamorphosed on the same day. Within ten days afterwards 127 normal larvae and six abnormal ones had metamorphosed to young sea-urchins from this culture. MacBride (**11**, p. 294) got the larvae of Echinus esculentus to metamorphose in forty-two to fifty days after fertilization, while Allen and Nelson (**1**, pp. 420–1) found the earliest metamorphosed young of E. acutus forty-two days after fertilization, of E. esculentus, forty-eight to sixty-eight days, and of E. miliaris, thirty-eight days. As compared with these records of regular sea-urchins our case was much quicker in development. On the other hand, the culture no. 11 and others from the same parents suffered from want of food seriously after the first week of their development, and when examined on September 3 they were, though seventy-six days old, all very far from metamorphosis, the 'larval' body fully developed, but the echinus-rudiment, if present, being very small. The culture no. 6, for some unknown cause, gave poor results. Most of the larvae died off very quickly, and the survivors showed various irregularities in shape.

The food supply was generally good during the first week or so, but afterwards in most cases it could not be continuous, and became unavoidably very irregular, owing to the unsuccessful culture of Nitzschia.

Now, from among the 'treated' larvae (nos. 4 and 9), which number 784 in all, there were found 88 inverse (11·2 per cent.) and 6 doubles (0·8 per cent.). In 'controls' (nos. 1, 6, and 11), on the other hand, from among 646 larvae, there appeared 69 inverse (10·7 per cent.) and 13 doubles (2 per cent.). This shows clearly that there is no noticeable difference in the rate of producing abnormalities between these two differently treated lots. We shall discuss this question later on (p. 143).

The results of Professor MacBride's experiments of producing the double hydrocoele (**15**) may here be cited briefly.

1914 (pp. 334–5). The larvae three or four days old were treated for ten or eleven days with 'hypertonic' sea-water

Table showing the Results of Experiments.

Culture No.	*Provisional name of culture.*	*Finger-bowl Period (beginning at the time of fertilization, kept in 'outside water').*	*Breffit-jar Period (feeding on* Nitzschia *begun).*	*Plunger-jar Period*[1] *(water consisting of synthetic sea-water mixed with a small quantity of 'outside water').*	*Total number of larvae examined.*	*Number of larvae with inverse situs.*	*Number of larvae with double hydrocoele.*	*Number of*[2] *larvae devoid of hydrocoele.*
1	'Control'.	May 20–May 21 (1 day).	May 21–June 1 (11 days), still kept in 'outside water'.	June 1–June 7 (6 days).	450	46 (10·2%)	1 (0·2%)	Few.
4	'Treated'.	Do.	May 21–June 1 (11 days), treated with 'hypertonic' sea-water for five days, May 22–May 27.	Do.	334	54 (16·2%)	0	16 (4·8%)
6	'Control'.	May 21–May 22 (1 day).	May 22–June 4 (13 days), still kept in 'outside water'.	June 4–June 7 (3 days).	30	1 (3·3%)	8 (26·7%)	Few.
9	'Treated'.	Do.	May 22–June 4 (13 days), treated with 'hypertonic' sea-water for five days, May 22–May 27.	June 4–June 5 (1 day).	450	34 (7·6%)	6 (1·3%)	Few.
11	'Control'.	June 19–June 20 (1 day).	June 20–June 23 (3 days), still kept in 'outside water'.	June 23–Sept. 3 (72 days).	166	22 (13·25%)	4 (2·4%)	Fairly many.

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[1] The end of this period means the time when the larvae were examined and recorded as in the adjoining columns. They were kept alive further, being either put back into the same jar as before, or transferred to Breffit jars until some of them passed metamorphosis.

[2] Except in no. 4 the exact number of such larvae in each culture was not counted. In nos. 1, 6, and 9 the percentage did not seem much different from that given for no. 4.

which had been prepared by evaporating. A right hydrocoele appeared but the amniotic invagination failed to appear, and the larvae refused to develop further.

1915 (p. 335). From among the larvae treated as above the most promising ones were isolated and fed on abundant Nitzschia. One larva produced a five-lobed hydrocoele on the right side.

1916 (p. 335). In both groups, those kept throughout in ' hypertonic ' sea-water and those put back in normal sea-water, after being treated for one to three days, were found some larvae with an unmistakable right hydrocoele provided with five tentacles.

1917 (pp. 335–7). ' Hypertonic ' sea-water was prepared this time by adding common salt to sea-water. The fourth-day larvae were transferred to ' hypertonic ' sea-water and allowed to remain in it for six days, after which period they were again put back in normal sea-water. The larvae with double hydrocoeles were about 2 per cent. in one jar, while at least 5 per cent. were in the other. Amongst hundreds of controls there was found only one specimen which had a double hydrocoele.

The result obtained in 1919 was so similar to that of the foregoing year that he thought it unnecessary to publish anything about it.

Before further discussing the causes and processes of formation of the abnormalities, let us stop for a moment to consider some questions which may naturally arise in the reader's mind. These are the questions of fundamental importance: (1) Is not the writer's discovery due to an error of observation? (2) Is not the occurrence of such abnormal larvae also common in nature for this particular species—at least in a particular season and at a particular place? (3) Is not the scantiness of records due to negligence on the part of previous observers? (4) Is not the so-called ' abnormal ' condition hereditary?

(1) It is rather incredibly frequent to find that even careful observers make an error in the use of the so-called endless screw of the fine adjustment of some microscopes so as to confound the upper surface of the object with the under

surface, for instance, with the result that a minute spiral structure may be taken as turned in a wrong direction. In my case it will be quite sufficient to state that as the larvae were fairly large objects under the microscope, I used to focus by means of the coarse adjustment while examining them with respect to the symmetry relations.

(2) It is now impossible to compare our culture with the larvae belonging to the same species which might have been found in plankton near Plymouth in the early summer of the same year (1920). One may suppose that if quite a number of naturally-developed larvae were examined carefully there might also be found some such abnormal forms. I think one may safely say, however, that at least the occurrence of this abnormality in so high a percentage as more than 10 per cent. is really due to artificial conditions.

(3) In view of the fact that in our cultures such larvae with inverse situs were eight times as numerous as the doubles (157 : 19), I cannot help doubting that the previous workers, who were fortunate enough to discover a few double-hydrocoele specimens from among hundreds of larvae, would have overlooked those inverse forms which might have been more frequent. It is very desirable to know if situs inversus occurs also fairly frequently in other species of sea-urchins when artificially reared.

(4) As stated above, the five lots of cultures shown in the table were obtained from three different parents. It is highly improbable that such a remarkable case, if inheritable, was found in at least three individuals out of seventy sea-urchins (more than 4 per cent.) which had been sent from Plymouth.

From all these considerations I am driven to conclude that the occurrence of the abnormality is true, and can even be fairly frequent among artificially-reared larvae.

4. Changes which may possibly have taken place during Earlier Stages.

One of the most remarkable and well-known cases of situs inversus among animals is that of the snails with sinistral shells. In some genera and species it is a normal character, while in others it is regarded as abnormal. As is well known, the sign of the reversal goes as far back as the segmenting egg, which shows its spiral cleavage in the direction contrary to that found in the eggs which will give rise to normal dextral snails. Conklin (**3,** p. 585) tried to interpret the phenomenon by assuming the reversal of the polarity in the egg, which change might have taken place in its very early stage. This hypothesis, though still lacking any satisfactory experimental evidence, is very simple and admirable ; and besides this we have as yet no other explanation.

There is no reason to deny that a state similar to that occurring among sinistral Gasteropods may occur also among Echinoderms. But can we not find in our cases of Echinus larvae any other interpretation which is more plausible and more probable than this ?

The Echinoderm egg has been known to be ‘ equipotent ’, or, in other words, the distribution of the organ-forming substances becomes established much later than in the eggs of most other groups. We owe to Runnström our knowledge of this question. In his series of experiments with Strongylocentrotus lividus (**24,** pp. 533–44, Text-figs. 7 *a*, 10) he showed that in this species embryos developing from half-eggs assumed normal characters later than did similar embryos of Echinus microtuberculatus and Sphaerechinus granularis. The larva developed ‘ probably ’ from the right half of the egg of Strongylocentrotus has its skeleton more strongly developed on the right side than on the left, and, moreover, the coelomic sac appeared only on the right side. Another of his experiments (**28,** pp. 471–3, Text-figs. 16 *a*, *b*) shows that when an early gastrula of Solaster sp. had been constricted along its median line, in the double monster so produced, no hydrocoele formed ; but a dorsal pore appeared on its left side instead of on the right, forming a mirror-image of the dorsal pore of the left half. He thus confirms what Driesch observed in some few double monsters of Echinus microtuberculatus in 1906 (**4**, p. 765). These results, considered in connexion with Spemann's Triton twins and double monsters and also with Morrill's double monsters of the trout referred to in a foregoing page (p. 106), lead us to expect that if successfully reared we might get an inverse larva from the right half of the egg in these Echinoderms also. Indeed, Spemann suggested this idea at the end of his work (**29**, p. 413). I may, however, only mention that our inverse larvae were all of normal size, and that there can be no doubt as to their having been developed from whole unseparated eggs. Gemmill's information of several cases of twin larvae of Luidia sarsi (**6**) is not uninteresting in this respect. Eggs of early cleavage stages were sent from Plymouth to Glasgow, and, according to him, the long-continued shaking during the transportation might have caused the blastomeres to dissociate and such twins resulted. His figures, especially of those 'side-by-side' doubles (Pl. ii, fig. 13; Pl. iii, figs. 19, 21), clearly show that there is no perceptible difference in structure between the two halves developed from partially-separated blastomeres, nor is there any sign in the right half of assuming a mirror-image of the left. We cannot, however, help doubting whether separation really took place during the long-continued shaking. Judging from the haphazard relative positions of the halves and from apparent differences in age between them in some cases, one may naturally suspect that the conditions observed resulted from fusion of two individuals. It is desirable to learn how the left side of a member will affect the right side of the other in artificially-grafted larvae. Results of both chemical (Goldfarb) and mechanical (Runnström) grafting of the eggs or embryos are unfortunately inadequate to solve the present problem.

5. Variations among Double-Hydrocoele Larvae and other Abnormalities.

Our attention will naturally turn to the double-hydrocoele larvae which appeared in cultures associated with the reversed larvae. To try to find if any relation exists between these two kinds of abnormalities we may first examine those known cases of double-hydrocoele and other abnormal larvae, and then consider the behaviour of individual organs and the interrelations to be found between them.

I. Hydrocoeles formed on both sides.

(*a*) Right hydrocoele and its associated structures more or less incomplete.

[Case 1.] Strongylocentrotus lividus. Runnström, 1912 (**23**), pp. 3–5, ‘no. 2’; 1918 (**26**), pp. 417–18, Taf. xiii, figs. 8 *a, b*. Reared at Monaco.

Left: anterior coelom large, divided into three regions: first, the ampulla to which the stone-canal opens; second, the main body of the axial sinus extending transversely to the right and communicating with the third region, the madreporic vesicle. The last-named vesicle exhibited no pulsating movement. Pore-canal and dorsal pore lacking. Stone-canal and hydrocoele well developed, the latter produced into five lobes. Amniotic invagination deeper than normal.

Right: anterior coelom smaller than that of the left, with pore-canal given out towards the epidermis, without, however, an opening to the exterior. Stone-canal showing a sign of degeneration, its anterior end beginning to be absorbed. Hydrocoele smaller than that of the left side. Amniotic invagination did not form on this side. Posterior coelom produced into an anterior process, which probably corresponds with genital stolon.

[Case 2.] Strongylocentrotus lividus. Runnström, 1912 (**23**), pp. 5–7, ‘no. 3’; 1918 (**26**), pp. 413–14, Taf. xiii, fig. 4. Reared at Monaco.

Left: anterior coelom large, consisting of two regions, one on the left, connected with stone-canal, the other on the right, corresponding with madreporic vesicle. The latter became later separated from the former, and was not seen pulsating. Pore-canal absent. Stone-canal and hydrocoele well developed, the latter produced into five lobes. Amniotic invagination formed but remaining totally undifferentiated.

Right : anterior coelom smaller than that of the left side. A vesicle was seen to be produced from it, which latter the author interpreted with some doubt as the hydrocoele. All these parts were seen beginning to degenerate. Amniotic invagination formed very late, but soon disappeared. Pedicellariae not formed. The posterior coelom produced an anterior diverticulum, probably representing the genital stolon.

[Case 3.] Strongylocentrotus lividus. v. Ubisch, 1913 (**30**), pp. 440–3, Text-fig. U. Reared at Naples (Giesbrecht).

Left : axial sinus well developed with the pore-canal which opened externally by a dorsal pore. Madreporic vesicle (' dorsal sac ') large, lying close to the axial sinus, but no communication between them existing at all. Echinus-rudiment fairly advanced. Genital stolon developed.

Right : axial sinus smaller than that of the left side, pore-canal only represented by a knob from wall of the former, and a fibrous tissue connecting the epidermis with this knob. Echinus-rudiment less advanced than that of the left side.

[Case 4.] Strongylocentrotus lividus. Runnström, 1918 (**26**), pp. 418–19, Taf. xiii, figs. 9, 10. Reared at Monaco.

Left : axial sinus, stone-canal, and hydrocoele all developed normally. Pore-canal and dorsal pore present, the latter later shifted its position towards the median line. Amniotic invagination formed.

Right : axial sinus well developed, with pore-canal and dorsal pore. The latter like its left fellow changed its position later towards the median line. Stone-canal formed later, its slight expanded posterior end representing the hydrocoele. No amniotic invagination formed.

[Case 5.] Echinus miliaris. MacBride, 1918 (**15**), p. 347, Pl. v, fig. 9. Reared in London.

Left : axial sinus fused with that of the right side and communicated with the exterior through a single pore-canal. Echinus-rudiment large.

Right : Echinus-rudiment smaller than the left one. No pedicellariae formed.

[Case 6.] Echinus miliaris. MacBride, 1918 (**15**), pp. 339, 343, 347, Pl. vi, fig. 11. Reared in London.

Left : axial sinus provided with a pore-canal and dorsal pore. Lobed hydrocoele and amniotic invagination developed normally.

Right : axial sinus with a pore-canal and dorsal pore. Hydrocoele smaller than that of the other side and no lobes were formed. Amniotic invagination absent. Two pedicellariae developed.

[Case 7.] Echinus miliaris. MacBride, 1918 (**15**), pp. 338, 339, 348, Pl. viii, figs. 18, 19. Reared in London.

Left : axial sinus fused with that of the right side. Madreporic vesicle situated between the compound axial sinus and the gut. Pore-canal and dorsal pore single. Echinus-rudiment well-developed. Stone-canal double, probably formed by the splitting of the string which had connected the hydrocoele bud with the anterior coelom.

Right : echinus-rudiment developed but, judging from the figures, it was smaller than the left one.

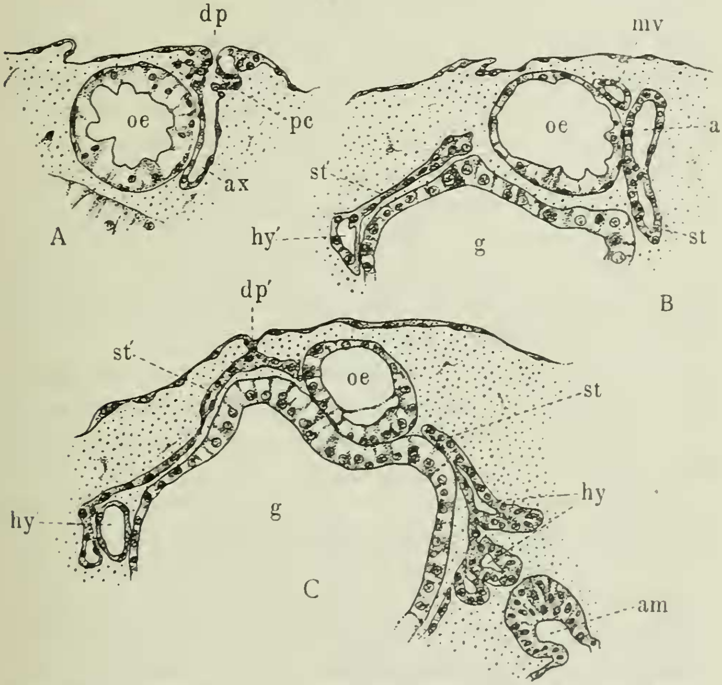
[Case 8.] Echinus miliaris. Culture 9, 'treated'. The larva was fifteen days old when found and killed.

Left : axial sinus well developed with a pore-canal and dorsal pore. Madreporic vesicle rather rudimentary, but distinctly seen lying close to the pore-canal. Stone-canal normal, leading to the slightly-lobed hydrocoele. Amniotic invagination formed.

Right : axial sinus and pore-canal rudimentary leaving no visible lumen. Dorsal pore absent. No madreporic vesicle found on this side. Hydrocoele as large as that of the left side, but simply vesicular in shape. Stone-canal has in its posterior part a distinct lumen and its calibre is as thick as its left fellow ; but the canal passes into a solid cell-string as it goes dorsad towards the vestigial axial sinus. No amniotic invagination formed.

(*b*) Left hydrocoele and its associated structure more or less incomplete. Such is found very rarely, and hitherto any definitely-recorded case belonging to this category is lacking. By the kind permission of Professor MacBride I examined his preparations and found among twenty whole mounts only a single specimen with the right echinus-rudiment larger than that of the left side. [Case 9.]

TEXT-FIG. 3.



Transverse sections of a fifteen-day-old double-hydrocoele larva of Echinus miliaris, in which the water-vascular system of the left side has begun to degenerate. ×300.
am, amniotic invagination ; *ax*. axial sinus ; *dp*, *dp'*, dorsal pores ; *g*, stomach ; *hy*, *hy'*, hydrocoeles ; *mv*, madreporic vesicle ; *oe*, oesophagus ; *pc*, pore-canal ; *st*, *st'*, stone-canals.

[Case 10.] Echinus miliaris. Culture 9, 'treated'. This larva was found to have double hydrocoele when fifteen days old, and then killed and examined by means of sections (Text-fig. 3).

Left : Axial sinus quite reduced, being represented by a solid thickening at the dorsal end of the stone-canal, while the latter also has no visible lumen (*st'*). Pore-canal represented by a solid cell-mass (*pc'*). Hydrocoele simple and vesicular (*hy'*). No amniotic invagination.

Right: anterior coelom well developed (*ax*), with its external communication through a pore-canal (*pc*) and dorsal pore (*dp*). Madreporic vesicle with fairly distinct lumen (*mv*), with its wall in contact with the axial sinus, but no communication whatever existing between them. Stone-canal (*st*) formed normally, and hydrocoele (*hy*) well developed, being provided with five lobes. Amniotic invagination formed (*am*).

[Case 11.] Echinus miliaris. Culture 9, 'treated'. This was also fifteen days old when found and killed.

Left: no trace of axial sinus and pore-canal to be found. Stone-canal ending blindly at the anterior end, while posteriorly it opens to a small widened cavity of the hydrocoele. Amniotic invagination formed but small.

Right: axial sinus, pore-canal, dorsal pore- and stone-canal all well developed. Madreporic vesicle lying close to the pore-canal. Hydrocoele lobed. Amniotic invagination a little smaller than the left one.

(*c*) The hydrocoele and its associated structures of both sides equal in their state of development or nearly so.

[Case 12.] Spatangoid pluteus collected at Messina. Metschnikoff, 1884 (**17**), p. 64.

Echinus-rudiments with ambulacral feet and spines 'quite equally' developed on both sides.

[Case 13.] Mellita pentapora. Grave, 1911 (**9**), pp. 35–46, Text-figs. 1–3. Reared at Beaufort, N.C.

Axial sinuses, hydrocoeles, and amniotic invaginations of both sides very nearly equal in development and symmetrically arranged. Two pore-canals opened by a common dorsal pore situated on the mid-dorsal line.

[Case 14.] Echinus miliaris. MacBride, 1911 (**14**), pp. 237–41, Pl. xxiv, fig. 1. Reared in London.

Here also axial sinuses, hydrocoeles, and amniotic invaginations were found almost exactly in the same state on both sides.

There was only a single dorsal pore, and no trace of madreporic vesicle was found.

[Case 15.] Echinus esculentus. MacBride, 1911 (**14**), pp. 241–4, Pl. xxiv, figs. 2–4. Reared at Plymouth (de Morgan).

Echinus-rudiments fully developed when examined and drawn by the observer, the larva then being fifty-five days old. The echinus-rudiment of the right side very slightly smaller and less advanced than the left one. Two pedicellariae developed on the right side and a third appeared at the posterior end.

[Case 16.] Strongylocentrotus lividus. v. Ubisch. 1913 (**30**), pp. 440–3, Text-fig. T, Taf. viii, fig. 26. Reared at Naples (Giesbrecht).

Axial sinus well developed on both sides and almost the same in size, each beset with a pore-canal, which opened to the exterior separately by dorsal pores. Mid-dorsally-situated madreporic vesicle communicated through a narrow canal with the right axial sinus. Echinus-rudiments and stone-canals on both sides equally well developed.

[Case 17.] Echinus miliaris. MacBride, 1918 (**15**). pp. 338, 347, Pl. v, fig. 8. Reared in London.

Echinus-rudiments on both sides of almost equal size. Dorsal pores two, and no pedicellariae.

[Case 18.] Echinus miliaris. MacBride, 1918 (**15**). pp. 339, 347, Pl. vi, fig. 10. Reared in London.

Both echinus-rudiments nearly equal in size, the right one slightly smaller. Dorsal pore single. A single pedicellaria formed on the right side.

[Case 19.] Echinus miliaris. Culture 1, 'control'.

The larva when found was eighteen days old, and had a lobed hydrocoele, stone-canal, and amniotic invagination developed almost exactly in the same state on each side. Two pore-canals opened separately through a respective dorsal pore. The larva has been carefully fed on sufficient food, and the echinus-rudiments on both sides developed at equal rate. The two dorsal pores retained as before their side-by-side positions.

No asymmetry in shape of the stomach was to be found. The unpaired spine appeared at the hind end on the median line. The larva lived for forty-six days and was at the end of that period very near to metamorphose, but was missed suddenly, and hence no further information on the internal structures could be obtained.

[Case 20.] Echinus miliaris. Culture 9, 'treated'.

The larva was found and killed when it was fifteen days old. The flattened axial sinus, pore-canal, stone-canal, and lobed hydrocoele developed nearly symmetrically on each side. Only on the right side the pore-canal ended in a solid cell-mass, and no dorsal pore opened. Amniotic invaginations formed on both sides, the right one being smaller than that of the left side. No madreporic vesicle found.

(*d*) Two hydrocoeles formed on one side.

[Case 21.] Echinus miliaris. MacBride, 1918 (**15**), p. 339. Reared in London.

There were formed two hydrocoeles on the right side due to the splitting of the hydrocoele bud which had been formed at the hinder end of the anterior coelom. One of them normally developed and had associated with it an amniotic invagination. The other, smaller and situated posteriorly to it, also possessed well-developed lobes. There was, however, no amniotic invagination for the smaller hydrocoele.

II. Hydrocoele formed on the left side only as in normal larvae, but some abnormalities found in other associated structures.

(*a*) Amniotic invaginations on both sides.

[Case 22.] Strongylocentrotus lividus. Runnström, 1912 (**23**), p. 7, 'no. 4'; 1918 (**26**), pp. 414–15, Taf. xiii, figs. 5, 6. Reared at Monaco.

Left: anterior coelom extending along dorsal side to the right to form a canal, which had no external opening. It seems, however, that an opening existed in an earlier stage. Stone-canal ending blindly at the posterior end. Hydrocoele isolated, with five lobes, and a blind canal sent towards the stone-canal. The author suggests that this hydrocoele may probably have differentiated from the posterior coelom of the left side. Posterior coelom was absent at first, but appeared later. Amniotic invagination formed.

Right : anterior coelom posteriorly situated, being very elongated and developed much more strongly than normal. Despite the absence of hydrocoele on this side a small and shallow amniotic invagination appeared later.

[Case 23.] Strongylocentrotus lividus. Runnström, 1918 (**26**), pp. 415–17, Taf. xiii, figs. 7 *a, b*. Reared at Monaco.

Left : anterior coelom undifferentiated and devoid of external communication. Pore-canal ‘ post-generated ’ and dorsal pore opened on the mid-dorsal line. Hydrocoele at first remained undifferentiated, but later, when amniotic invagination appeared, it began to develop again.

Right : hydrocoele not formed. Amniotic invagination appeared later, but soon degenerated.

(*b*) No amniotic invagination formed.

[Case 24.] Strongylocentrotus lividus. Runnström, 1918 (**26**), p. 424, Taf. xiv, figs. 17 *a, b*. Reared at Monaco.

The left anterior coelom represented by a widened end of the stone-canal, and a short wide pore-canal opening externally. Hydrocoele provided with four lobes, but owing to the absence of amniotic invagination its development was abnormal. One of the primary tentacles gave out a small branch which corresponds to one of the paired tentacles.

(*c*) Pore-canal and madreporic vesicle doubled.

[Case 25.] Strongylocentrotus lividus. Runnström, 1918 (**26**), p. 419, Taf. xiii, fig. 11. Reared at Monaco.

Both axial sinuses beset each with a pore-canal opening to the exterior by a dorsal pore. A pair of vesicular organs lying each near the pore-canal of each side were identified without doubt by the author as madreporic vesicles. The one on the left side acquired later a communication with the left axial sinus, while the other on the right side began to degenerate.

III. Hydrocoele absent from both sides.

[Case 26.] Strongylocentrotus lividus. Runnström, 1918 (**26**), p. 424, Taf. xiv, fig. 18. Reared at Monaco.

Amniotic invagination failed to be formed on the left side; and, instead of it and at the place where the former should in normal case be formed, a calcareous spine appeared.

[Case 27.] Echinus miliaris. MacBride, 1918 (**15**), pp. 339–40, Pl. vi, figs. 12–14; Pl. x, figs. 22–3. Reared in London.

Under this heading more than one specimen will be described together. Anterior coelom on neither side enlarged so as to form an axial sinus. On neither side was a hydrocoele discovered, nor was there any vestige of a stone-canal or a dorsal pore. Only in an exceptional case was there found a dorsal pore. In one case a madreporic vesicle was seen and figured (fig. 22). A group of pointed spines developed on each side within the loop of the ciliated band and another spine was found situated dorsal to this loop on both sides.

6. Considerations on the Organs and Structures concerned and the Factors concerned in their Development.

(*a*) Anterior Coelom.—This is formed separately on each side pinched off from the posterior coelom, the left one being earlier in its formation than the right fellow (see MacBride, **11**, p. 298). Sometimes the two anterior coeloms unite to form a single sac on the dorsal side of the larval oesophagus (Cases 5, 7). The left one is connected with the pore- and stone-canals and remains as a distinct sac, called the axial sinus, while the right one normally remains as a simple sac and very often degenerates later.

(*b*) Madreporic Vesicle.—This is a minute round sac normally found a little on the left side lying close to the pore-canal, and often is stated to exhibit a rhythmic pulsation. MacBride (**11**, p. 299) discovered in Echinus esculentus that this vesicle was derived from the right anterior coelom, at first as a solid thickened end of a string of cells given out from the posterior end of this coelom. Later (**16**, pp. 261–2) he confirmed this in Echinocardium cordatum, in which species the vesicle in question is unusually large. Runnström found a pair of madreporic vesicles in a larva of Strongylocentrotus lividus (Case 25), and, moreover, according to him, the one on the left side became later connected with the axial sinus of the same side. Perhaps other instances of the presence of a communication between the vesicle and one of the axial sinuses (Cases B, 1, 2, 16) may also be due to a secondary change. In the Case 2 the vesicle is seen later again separated from the coelom. Often this vesicle is absent (Cases 14, 20). v. Ubisch (**30**, p. 443) is of the opinion that the madreporic vesicle was not possessed by the ancestor of the sea-urchins, but that it represented the only remnant of the degenerated right anterior coelom having assumed a new but unknown function in the course of phylogenetic development. And, further, according to him, when the right anterior coelom made its unusual development the highly-differentiated and functioning madreporic vesicle could not be affected thereby and both of them existed side by side.

In the reversed and also in some double-hydrocoele larvae (Cases 10, 11) the madreporic vesicle was found on the right side, close to the right pore-canal. In the case where two such vesicles are present (Case 25) the right one may be the homologue of this. In neither case is its origin made clear. From want of sufficient material and from our ignorance of its function any definite statement will be premature.

(*c*) Pore-canal and Dorsal Pore.—The primary dorsal pore is formed from the left coelomic sac to communicate with the exterior before the latter becomes divided into the anterior and posterior coeloms. In the course of further development of the larva the pore shifts from its original position on the left side towards the mid-dorsal line. This shifting is preceded by the formation of a transverse groove of the ectoderm. Probably in connexion with this shifting process it is often the case that the canal gets temporarily or permanently obliterated (Cases B, 1, 2, 20, 22, 23). The cause is unknown to us; still, I think there is hardly any doubt as to its being due to artificial conditions. Too large a number of diatoms or bacteria in the vessel in which the larvae have been kept may cause this. Shortly afterwards the pore and canal can regenerate (Cases B, 23) and the revived development of the whole water-vascular system follows. In other instances no second pore was formed, and degeneration of the system soon set in (Cases 1, 8, 10, 11).

The presence of the right pore-canal side by side with the left is a constant and normal character in the larva of Mellita pentapora (Grave, **9,** p. 42; and also his former paper, 1902, p. 58). The same is not common in Echinus miliaris; still, it has been recorded by MacBride in a larva which was otherwise quite normal (**15,** p. 339). Although the presence of two pore-canals is a very common occurrence among double-hydrocoele larvae (Cases 4, 6, 16, 17, 19, 20) it seems by no means to be a necessarily associated feature. In starfish the occurrence of the double dorsal pore has never been seen even among double-hydrocoele larvae (Gemmill, **5,** p. 230; **7,** p. 31; **8,** p. 62). To such an important difference found between these two classes let us return later (p. 142). According to Runnström the formation of the dorsal pore and pore-canal seems to be a self-differentiation (**25,** p. 301).

(*d*) Stone-canal.—This is the part which at first connected the hydrocoele bud with the main body of the anterior coelom. This canal is sometimes found doubled, being caused from either its defective origin (Case 7) or abnormal regeneration (Case B). When degeneration takes place, probably due to the lack of communication with the exterior, it begins from that end which is adjacent to the axial sinus (Cases 1, 8, 11).

(*e*) Hydrocoele.—It is a well-known fact that the right coelomic sac has in normal larvae the potentiality of producing a sac which is homologous with the left hydrocoele. Such a special organ-forming substance seems to be located especially at the place where the coelomic sac has to divide later into the anterior and posterior coeloms. We see from MacBride's work on Ophiothrix fragilis (**13**, pp. 578, 586) that this sac, homologous with the left hydrocoele, exhibits varying degrees of development among normal larvae, and in a few extreme cases it gives rise to a five-lobed hydrocoele (Pl. xxxvi, fig. 54; compare further those double-hydrocoele Ophioplutei described by Müller and Metschnikoff).

Whether this unusual development of the right hydrocoele is to be regarded as a case of atavism or as another kind of variation is a matter of choice. MacBride (**14**, pp. 240, 244) is of opinion that the free-swimming ancestor of the Echinoderm had a pair of hydrocoeles, equally developed on each side, the right one has, however, become atrophied as soon as the free-swimming habit was given up. The appearance in some abnormal larvae of a right hydrocoele is an atavistic feature. But, according to him, the appearance and further completion of the associated structures, such as amniotic invagination, set of spines and dental sacs, derived from the ectoderm and mesoderm respectively cannot be accounted for by atavism, because it is quite impossible to endow the ancestor with such a double set of highly-developed spines and Aristotle's lanterns. Therefore, he introduced the idea of the internal secretion, in that the abnormally-developed right hydrocoele must have given off some stimulating substances which caused both ectoderm and a part of the posterior coelom to respond, with the result that there appeared a second set of spines and dental sacs. He further discussed this theory in his second paper on the double hydrocoele (**15**, pp. 341–5). Some months earlier than the first of these papers Grave (**9**, p. 43) discussed the same idea and made the objection 'that such an explanation presupposes that the series of structures in question was present and in some way related in the normal development of the ancestral echinoderm, a supposition for which there is no basis in observed fact'.

Now, we may find no great difficulty in assuming that such stimulating power of the left hydrocoele has been acquired since the disappearance of the right hydrocoele, as v. Ubisch (**30**, p. 444) remarked in reply to Grave's objection. It is necessary, however, to introduce another supposition to understand how the right hydrocoele in our abnormal case acquired that power of stimulating other tissues, which power was not possessed by the right hydrocoele of the ancestor. In short, even if we accept the view that the Echinoderm ancestor possessed a double hydrocoele, it seems to me that the atavistic interpretation has to encounter with such a difficulty as stated above.

The development of a right hydrocoele to such an unusual degree may then safely be regarded as a case of homoeotic variation. The examples of this kind of variation given by Bateson (**2**, pp. 721–35) should be classified at least into two different groups. One group contains the cases characterized by the appearance on one side of a wholly new structure, which is quite unknown in the animal's phylogenetic history, whereas a mirror-image of it is normally present on the other side. Gemmill's 'primary' homoeosis (**8**, p. 71) seems to be this. A tadpole of Pelobates fuscus with a second spiracle on the right side is an example, and if Runnström's view is accepted the appearance by self-differentiation of an amniotic invagination on the right side of the sea-urchin larva would be another. The second group comprises those cases where, in obviously paired organs, one member, which is normally vestigial, develops in certain circumstances to the same degree as its fellow. A double-tusked narwahl is the best illustration of this kind. Gemmill's term 'secondary' homoeosis perhaps denotes the same phenomenon. I feel very doubtful whether the case of our double hydrocoele should be placed under this latter category or under the first. The paired origin of the front teeth in the narwahl is quite obvious, while the presence of a pair of well-developed hydrocoeles in the Echinoderm ancestor will not be accepted unanimously by all zoologists.

I do not believe that the development of a double hydrocoele has 'resulted in a larval organization better adapted to the conditions under which the existence of the pluteus is led', as Grave (**9**, p. 45) states in his discussion on the homoeosis.

We need not explain the cause of homoeosis in this way only. The chance by which the double hydrocoele is induced to develop seems to be quite unusual, as I will try to show presently. It is not at all a result of adaptation.

In his famous experiments on Alpheus, Przibram showed that if a large claw of this Crustacean is amputated a small claw will appear at the spot, whilst the small claw of the other side, which was not operated upon, will become a large claw. This phenomenon he calls ‘compensatory hypertypy’. For more detailed information I refer to his later paper (**22**). A similar but slightly different idea can be applied in the case of double hydrocoeles. The right hydrocoele might have arisen as a result of compensatory hypertypy caused by the arrested state of development in the left hydrocoele. The differences from the case with Alpheus are that (*a*) the presence of a rudimentary right hydrocoele is not a normal feature, but no doubt the right anterior coelom has a potentiality of producing it, while the small claw of Alpheus is present constantly and quite functional, and (*b*) the left hydrocoele has not yet been fully developed but arrested in its early stage of development, while the large claw of Alpheus was removed after it had reached the full-grown state. With these differences kept in mind we may use Przibram's term in our case as well.

According to Runnström (**25**, p. 305) the further differentiation of the hydrocoele, left or right as the case may be, depends largely on the formation of an amniotic invagination. There was, however, an exceptional case (Case 24). Besides, from lack of a corresponding amniotic invagination and from obliteration of the dorsal pore, the hydrocoele and its associated structures will degenerate from hunger (Runnström, **25**, p. 265; MacBride, **15**, pp. 339, 340).

The presence of two hydrocoeles on one side was noticed by MacBride (Case 21), and interpreted as being due to the splitting of the hydrocoele bud. Another curious abnormality was described by Runnström (Case 22). There are, according to this observer, two possibilities as to the cause of such an isolated hydrocoele : (*a*) it may have been separated from the end of the stone-canal, or (*b*) the posterior coelom may have given rise to it under the influence of the amniotic invagination. From the absence of posterior coelom, though one appeared afterwards, he thinks the latter more probable. In one of Runnström's larvae of inverse situs (Case B) we see another extraordinary feature in the right hydrocoele (**23,** p. 9 ; **26**, p. 423). The hydrocoele was three-lobed, and close to it there were two curious structures. One was a round closed vesicle, the origin of which the author could not ascertain. The other was an ectodermal groove running nearly parallel to the stone-canal and lined with very actively-moving cilia. This groove at last became separated from the ectoderm, and together with the above-stated closed vesicle, united with the hydrocoele, remaining as a larger lobe of the latter. Runnström is of the opinion that in those pathological cases a hydrocoele or a part of it can be formed both from posterior coelom and ectoderm.

(*f*) Amniotic Invagination.—This is formed some days later than the appearance of the hydrocoele. It seems to me highly probable that this structure is homologous with the stomodaeal invagination of Holothurians. As early as 1906 MacBride (**12,** p. 615) pointed out that the larval stomodaeum of Holothurians reminds one ' of the amniotic cavity in the Echinopluteus '. This idea has since found another support in the fact that in Cucumaria the stomodaeal invagination is formed to the left of the mid-ventral line, as was first discovered by Newth (**20,** p. 634, Pl. i, fig. 6) and afterwards confirmed by the writer (**21,** pp. 379, 384, Pl. v, figs. 5 and 6). It is therefore quite improbable that the ancestral Echinoid had a pair of amniotic invaginations. MacBride (**15,** p. 343) never found in any single instance an amniotic invagination formed where no hydrocoele existed, and confirmed his former view (**14,** pp. 240–1) that the undifferentiated ectoderm can give rise to an amniotic invagination only under the influence of the hydrocoele. Runnström's view is diametrically opposed to this. He has shown us several instances where an ectodermal invagination was formed at a place under which no hydrocoele had been developed (Cases B, 22, 23, and also **25,** p. 271). He further made experiments to prove his view that the formation of the amniotic invagination is a self-differentiation and is not formed from stimulus of an underlying hydrocoele. He could produce a new amniotic invagination in a larva of Echinus miliaris from which the echinus-rudiment had been removed (**27,** pp. 9–11). In another of his experiments an amniotic invagination was seen to appear in each of two pieces of a larva where the normally-formed invagination had not been included; thus in this larva three amniotic invaginations in all were formed (pp. 13–14). It may be mentioned that in all of his cases the ectodermal invagination was very small and lined with flat epithelial cells. In another place he states (**25,** p. 302) that the invaginated ectoderm forms cylindrical cells only at the place where the hydrocoele wall comes to be in contact, while in the other part the cells remain flat. I myself understand by the term amniotic or ‘ echinid ’ invagination an ectodermal pit whose epithelial cells are from its first appearance high and cylindrical, even when fairly apart from the hydrocoele (Text-figs. 2, D, and 3, C, *am*). In this sense I cannot help doubting whether all of Runnström's structures deserve the name amniotic invaginations. He admits that the further development and differentiation of the amniotic invagination is conditioned by the presence of a hydrocoele, and that without it the former degenerates (**25,** p. 305). It is of interest to see that he pointed out that the rôle of an amniotic invagination could be played, to a less extent, by other ectodermal invaginations, such as that which he termed ‘ spine invagination ’ (Case B). According to him, if there was no amniotic invagination formed the stone-canal stopped developing when it had reached its normal length, and later gradual degeneration set in of the whole water-vascular system. But, as for the larva in question, the ‘ spine invaginations ’ were situated further back than normally an amniotic invagination is placed, and the stone-canal did not stop at the normal length, but continued to lengthen until the hydrocoele reached those invaginations (**26,** p. 421). As to the nature of these invaginations let us examine again (p. 138).

(*g*) Posterior Coelom and Genital Stolon.—The anteriorly-prolonged end of the left posterior coelom shares the formation of the echinus-rudiment (MacBride, **11,** pp. 304–5). This change takes place also on the right side in abnormal larvae where a right hydrocoele developed. In the normal case the genital stolon makes its appearance shortly before metamorphosis from the wall of the left posterior coelom (MacBride, **11,** p. 309). How its right fellow behaves in abnormal larvae is still an open question. Runnström inclines to think that in two of his double-hydrocoele larvae (Cases 1 and 2) a rudiment of genital stolon was formed from the right posterior coelom. v. Ubisch (**30,** p. 445) concludes that the doubleness is not extended to all organs as shown from the fact that in his older double-hydrocoele larva (Case 3) the genital stolon was seen formed only on the left side. This conclusion cannot pass unchallenged because in this larva the right echinus-rudiment was much less advanced than the left, and also because the structure in question is not distinct until the larva reaches the height of its growth.

(*h*) Pedicellariae.—In normal Echinus larvae there appear a pair of pedicellariae on the right side, one being dorsal to the loop of the ciliary band, the other ventral to the same. In some imperfectly symmetrical double-hydrocoele larvae one or both of them appear on the right side only (Cases 6, 15, 18) or on both sides of the larva (MacBride, **15,** p. 343). According to Runnström the reversed larvae of Strongylocentrotus had pedicellariae appearing on the left side (Cases A and B), and I am inclined to believe that it is also the case with our Echinus, though unfortunately any positive evidence is lacking at present. In the complete absence of hydrocoele from both sides no true pedicellariae appear (Case 27). Thus the relation between the pedicellariae and echinus-rudiment (or hydrocoele) is somewhat complicated. Probably the echinus-rudiment calls forth the formation of pedicellariae on the opposite side. It seems to me that they are not inhibitory to each other on the same side, because they can co-exist side by side. The fact, however, that in most of the double-hydrocoele larvae the pedicellariae are not formed may simply be due to lack of sufficient material, or that the echinus-rudiment, being more vigorous in development than the pedicellariae, wins the competition. MacBride assumes that 'the influences emanating from a hydrocoele not only tend to inhibit the formation of pedicellariae on the same side but to determine their formation on the opposite side of the larva' (**15**, p. 343), and that the hydrocoele can act as such even in its early stage. Thus, the fact that an echinus-rudiment and a pedicellaria or two can co-exist on the same side is explained by him in the following manner: 'If we assume that in these larvae the growth of both hydrocoeles has been arrested at an early stage, but after the stage at which the stimulus to form pedicellariae on the opposite side had already gone forth from them, and that then, after the formation of these organs on both sides had been determined, further nourishment became available and the left hydrocoele developed further, the structure of such larvae can be explained' (pp. 343–4). Runnström's case that some starved larvae, which had no hydrocoele, developed a pair of pedicellariae (**25**, pp. 269–70, Text-figs. 33–5) is now very difficult to understand. It is doubtful whether the hydrocoele was really absent in those larvae.

(*i*) Spine.—The larva of Echinus miliaris produces, when fairly grown, a rudiment of a spine at the hind end a little towards the right from the median line. This gives rise, as do some others which develop later, to a square-ended spine on the future abactinal side. This rudiment is found situated a little on the left side in reversed larvae (Text-fig. 1, sp_1), and in most of the double-hydrocoele larvae, in an almost median position. Such a different position of this spine is undoubtedly correlated with the different behaviour of the echinus-rudiment. Characteristic are the spines which develop in the larvae devoid of hydrocoele (Case 27). As already stated there is a group of pointed spines and a solitary one on each side of the larva. Runnström found such a spine only on the left side (Case 26). I am much inclined to think that from want of regulating influence of the hydrocoele the rudiments of pedicellariae were developed in an aberrant way into some of those peculiar spines.

Runnström (**27,** pp. 21–2, figs. 21–3) discovered in the normal larva of Echinus miliaris a pair of small ectodermal invaginations formed inside the loop of the ciliary band on the right side. In each of these invaginations 2–3 spines belong to the Basalia 3 and 5 are later formed. He called the former 'spine invaginations' (**26,** p. 420). Spines undoubtedly identical with these have been seen by me on the left side of one of the reversed larvae (Text-fig. 1, sp_2). In Strongylocentrotus lividus these structures do not appear normally, still Runnström identified the pair of pits found in an abnormal larva with them (Case B). These may be an abnormal amniotic invagination divided into two. His descriptions and figures (**23,** p. 8; **26,** pp. 420–1, Taf. xiv, fig. 13) are not quite satisfactory enough to substantiate his refusal to look on them as modified amniotic invaginations.

(*j*) Gut.—We know really nothing about the change the gut undergoes in accordance with the formation of the double echinus-rudiment or situs inversus. Normally the definitive stomodaeum appears at the centre of the floor of the epineural space (MacBride, **11,** p. 307), and the rudiment of the oesophagus, as an outgrowth from the left wall of the stomach meeting the stomodaeum, appears later (p. 310). The adult mouth breaks through some days after metamorphosis, and the anus is formed still later (pp. 311–12). Runnström (**24,** pp. 544–52) found in the larvae developed from the eggs which had been treated with potassium-free sea-water some asymmetrical distortions in the larval stomach and formation of a new oesophagus on the left. He interpreted the phenomenon as the formation of the definitive oesophagus precociously indicated. It is quite conceivable that in the course of the development of an echinus-rudiment, no matter on which side of the larva it may lie, the hydrocoele, working together with other ectodermal and mesodermal tissues, can induce this new structure to appear and thus the actinal part of the young sea-urchin be completed.

7. PROBABLE MECHANISM WHEREBY ABNORMALITIES ARE PRODUCED.

From those observed facts above considered the following conditions seem to concern the production of abnormalities of the hydrocoele and its associated structures.

1. Obliteration of the pore-canal. This seems to be a cause of the arrest of the further development of the water-vascular system and then a quick degeneration of the whole system follows.

2. Activation of the right anterior coelom of its latent potentialities of producing a hydrocoele, to compensate the degenerating left hydrocoele.

3. Regeneration of the pore-canal or fusion of the two axial sinuses. Both afford the left hydrocoele a renewed communication with the exterior, and the further development and differentiation of the water-vascular system thereby take place.

4. Development of a right amniotic invagination and the peculiar change of the anterior prolongation of the right posterior coelom. These changes seem to have been evoked by the stimulus of the unusual right hydrocoele. These three elements working together give rise to an echinus-rudiment.

From these data, if adequately combined, the following changes are quite possible.

Let us start from a young normal larva, in which hydrocoele, axial sinus, pore-canal, and dorsal pore are all formed on the left side. An amniotic invagination may already be formed on the left side. The right anterior coelom may have a pore-canal.

Now, the dorsal pore of the left side becomes obliterated, which fact is followed by the arrest of development and further degeneration of the left water-vascular system. Two courses are here open : A. The right anterior coelom begins its unusual development to produce a right hydrocoele, which acquires communication with the exterior through a pore-canal. B. The right anterior coelom does not become active either from very weak disposition of the right anterior coelom or, more probably, from want of sufficient nutrition. The result is the total absence of hydrocoele from both sides.

The further fate of larvae in which the course of events has been that indicated by A will be one of the following three :

1. Appearance of a new dorsal pore on the left side which revives the power of the left hydrocoele to develop further. If well fed the hydrocoele on each side will continue to develop side by side so as to give rise to a double-hydrocoele larva.

2. Axial sinuses of both sides come in contact with each other and then unite, thus making the left hydrocoele regain its communication with the exterior and enabling it to develop further. The result is also a double hydrocoele.

3. No reappearance of a second dorsal pore nor fusion of the axial sinuses takes place. The left water-vascular system will then degenerate quickly, while the right one will develop like the normal left. A larva with situs inversus is the result.

In both the courses of events indicated by 1 and 2 the following three conditions may possibly arise, according to the different stages at which the right hydrocoele had arrived, when the recovery of the left hydrocoele took place :

(*a*) The recovery of the left hydrocoele takes place before the right hydrocoele attains a size equal to the left. The period during which the hydrocoele is deprived of communication with the exterior is very short. Under such a condition the result is a larva whose left hydrocoele or echinus-rudiment is larger or more advanced than that of the right side. This is very frequently met with among double-hydrocoele larvae.

(*b*) The left hydrocoele recovers at the time when the right one attained a size about equal to it. The larva developed under such a condition has two hydrocoeles or echinus-rudiments equal in size. Such a case is less frequently met with than the former.

(*c*) The left hydrocoele recovers late when the right one is in a more advanced state than it. The period during which the hydrocoele is deprived of communication with the exterior is here very long. The result is that the larva has the left hydrocoele or echinus-rudiment smaller than the right. Usually the hydrocoele and its associated structures cannot remain unchanged for so long a time after being deprived of its external communication. This case is therefore met with very rarely.

The above may not be the only ways of reaching the respective results, but probably are the commonest. Many modifications are naturally conceivable: for instance, the right dorsal pore may be obliterated in its turn, which causes the degeneration of the whole water-vascular system of the right side and thus a normal larva will result secondarily (see Case 8).

Let us now compare this interpretation of the occurrence of the inverse situs in Echinus larvae with Spemann's case of Triton larvae (**29**, p. 407). Though equally caused by a 'defective' development of a single organ—alimentary canal in Triton and hydrocoele in Echinus—further results in which the other organs become affected are different in these two cases. Instead of displacement of other adjoining organs, the arrest in development of the left hydrocoele causes a new hydrocoele to appear on the other side and also a new set of associated structures as a consequence. The normal left hydrocoele can, if it regains its opportunity of further development, produce another echinus-rudiment, so as to give rise to a double-hydrocoele larva. Any parallel of such a feature is very improbable in Triton larvae.

There is no reason to expect that the above is equally applicable to the formation of double hydrocoele of other classes of Echinoderms. Conditions may be totally different. Let us, for instance, take the case of the double-hydrocoele larvae of starfishes. Normally in most species of starfishes the paired coelomic vesicles grow forwards, and their anterior ends meet and unite in front of the larval mouth. The presence of two dorsal pores is very common, but the right one gradually atrophies (Gemmill, **5**, p. 231), and still the right coelomic vesicle retains its communication with the exterior through the left dorsal pore. The hydrocoele becomes later differentiated from the middle portion of the spacious left coelomic sac. In the case of the double hydrocoele the right one is likewise formed from the middle portion of the right coelomic sac. Among the double-hydrocoele larvae of Porania pulvillus and Asterias rubens Gemmill found no case of the presence of double dorsal pores, in all instances the left pore only being present (**5**, p. 230 ; **7**, p. 43 ; **8**, pp. 62, 69). Thus it is evident that the obliteration of a dorsal pore has hardly any influence on the further development of the hydrocoele on the same side. Under such a different condition I suppose that the occurrence among starfish larvae of the situs inversus as we find in Echinoid larvae will be extremely unusual. Gemmill tried to explain the cause of the double hydrocoele chiefly by the supposition that, owing to the over-fed condition of the larva, its stomach becomes expanded and globular, so that the ventral horn of the left posterior coelom tends to fail to unite with the right middle coelomic region. The latter region, being thus left isolated from the posterior coeloms, produces a right hydrocoele (**5**, p. 244 ; **8**, pp. 54–5). This interpretation in its turn cannot hold true in the case of those double-hydrocoele Echinoid and Ophiuroid larvae, in which no such extension of the left posterior coelom takes place normally (MacBride, **15**, p. 326). The discovery by MacBride (**10**, pp. 368–70) of a double-hydrocoele larva in Asterina gibbosa, in which species the egg is heavily laden with yolk, is a serious objection to the hypothesis of excessive food. One feature is, however, certainly common in the double-hydrocoele larvae of the three different classes : namely, the temporary arrest in the development of the left hydrocoele in some way or other in an early stage. And this occurs more frequently under artificial conditions than in nature.

With regard to the occurrence of the reversed Auriculariae, as discovered by Müller (**19**, pp. 101, 109, Taf. v, fig. 1), the attempt to interpret the phenomenon by virtue of the compensatory hypertypy is nearly hopeless. It is a widely-accepted fact that in Holothurians the right anterior coelom does not exist at any stage throughout life, whilst the hydrocoele is differentiated even before the coelomic sac divides into right and left halves (posterior coeloms). It is not easy to imagine that the right posterior coelom could ever produce a hydrocoele, when the normal hydrocoele happened to be arrested in its development. If this cannot be the case we must regard it as a result either of the change of polarity in the egg (according to Conklin, **3**) or of twin formation (of Spemann's sense, **29**).

8. External Factors as Causes of Abnormalities.

From series of his experiments MacBride (**15**) came to the conclusion that the chief cause producing double-hydrocoele larvae of Echinus was the increased salinity of the water used for culture. Unfortunately, as I have pointed out in a foregoing page (p. 114), the result of our experiments of this year was quite different from our expectations. As shown in the table the number of double-hydrocoele larvae was greater in 'controls' than in 'treated', i.e. 2 per cent. and 0·8 per cent. respectively. As the double hydrocoele and situs inversus start, I believe, under the same condition, the figures of reversed larvae may also be used in this connexion. The occurrence of the reversed larvae was practically equal in both 'controls' and 'treated', i.e. 10·7 per cent. and 11·2 per cent., the difference being within the range of probable error.

Let us now turn to examine whether artificial synthetic sea-water had anything to do with the production of abnormalities. Culture 11 came into contact with the synthetic sea-water when the larvae were four days old, Culture 1 when twelve days old, and Culture 6 when fourteen days old. They were examined and counted seventy-two, six, and three days afterwards respectively. Though it is unsafe to draw any decided conclusion from such few cases and numbers one can hardly see any effect of the synthetic sea-water on the production of doubles or reversed if allowed to act earlier in one culture than in others.

One might reasonably expect that the artificial treatment of the egg and sperm might have caused some disturbance from the normal development of the larva. This is of course quite possible, but I may only mention that it is curious to see that among such material as the sea-urchin egg so commonly used for study and demonstration in embryological work only a very few cases of the abnormalities in question have been noticed.

One of the most important factors which differ more or less from the conditions in nature is the food supply. The method of feeding marine larvae on diatom cultures, through which many different forms of pelagic larvae have been successfully reared, is relatively a recent introduction. The result is very often over-feeding. In an over-fed larva hypertrophy and other disturbances in growth is quite conceivable. From uneven distribution of food in the culture jar and from a different state in the health of larvae, over-fed and under-fed individuals may arise within one and the same jar. The obliteration of the normally-formed left dorsal pore, which seems to me a direct cause of the production of the double hydrocoele and situs inversus of the Echinus larvae, may be associated with the excess of diatoms and other minute organisms in the jar. Whether it is physiological or mechanical it is hard to decide at present.

Runnström (**25**, pp. 321–2) found that the larvae of Strongylocentrotus showed the degeneration of organs when over-fed on yolk. The echinus-rudiment was above all the most sensitive to the treatment and degenerated completely. Undigested yolk granules were found migrating everywhere, even scleroblasts were laden with them and the absorption of calcareous bodies followed. The effect of over-feeding on diatoms will naturally be very different from this. Though somewhat difficult to control (MacBride, **15**,

p. 338) it is desirable to experiment on the effect of different amounts of diatom-food upon the development of the larval organs.

The effects of hunger were observed both by Runnström (**25**, pp. 254–321) and MacBride (**15**, pp. 339–40). The difference between the results of these two observers is remarkable. In every instance of Runnström's larvae showed extreme degeneration of skeletons, while in MacBride's case the larval arms were almost normal, owing to the well-developed state of the skeletons, but the hydrocoele degenerated and peculiar spines formed. Besides the differences in degree and duration of hunger, the stage at which the larvae were treated, &c., there must be still other complicated factors which caused such different results. For those starved larvae bacterial infection is no doubt another important cause of abnormal development (**25**, pp. 273–4). Grave (**9**, p. 36) remarked that among the larvae of Mellita only those well fed developed the echinus-rudiment.

As to the effect of other chemical and physical environments upon the development of the sea-urchin larvae we have those valuable results obtained by Vernon, Tennent, and others. But we know hardly anything with regard to the changes of coelomic vesicles and hydrocoele treated specially.

9. Summary and Conclusion.

1. Under artificial conditions more than 10 per cent. of the larvae of Echinus miliaris exhibited the situs inversus.

2. So far as I could examine, the internal as well as external structures of such abnormal larvae were mirror-images of those of the normal larva.

3. The young sea-urchins metamorphosed from such inverse larvae showed no abnormal features externally.

4. The manner in which such abnormal larvae departed from the normal development seems to be analogous to that in the case of 'compensatory hypertypy' in the claws of Alpheus.

5. In an early stage of the normally-developing larva it

happens sometimes that the left dorsal pore becomes obliterated. This seems to be associated with the shifting of the pore towards the mid-dorsal line. The hydrocoele, thus deprived of its communication with the exterior, ceases to develop and then degeneration of the whole water-vascular system sets in.

6. The right anterior coelom, on the other hand, is now evoked to realize its latent potentiality of producing a hydrocoele (homoeosis). The degenerating left hydrocoele gives place to a newly-appearing right hydrocoele.

7. The right hydrocoele stimulates its adjoining tissues to give rise together to an echinus-rudiment.

8. The external factor or factors which cause the obliteration of the dorsal pore could not be found. This probably is connected with the presence of too much diatom-food and other micro-organisms in the culture jar.

9. If a new dorsal pore is formed on the left side before the degeneration of the left hydrocoele sets in, the developing power of the latter will thereby be revived. If sufficiently fed a double-hydrocoele larva will result under such a condition.

10. If, while the left hydrocoele is arrested in its development and then degenerates, the right anterior coelom fails to develop a new hydrocoele presumably from want of sufficient food, a larva devoid of hydrocoele will result.

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11. Appendix.

To the examples of the reversed larvae (p. 112) a case in the starfish Cribrella oculata as figured and described by A. T. Masterman is to be added (see 'Trans. Roy. Soc. Edinburgh ', vol. 40, 1902). My thanks are due to Professor J. F. Gemmill, who has kindly called my attention to this paper. Compare also: Gemmill, " Notes on the Development of the Starfishes Asterias glacialis O. F. M.; Cribrella oculata (Linck) Forbes; Solaster endeca (Retzius) Forbes; Stichaster roseus (O. F. M.) Sars ", 'London Proc. Zool. Soc.', 1916, p. 557.

With regard to the reversed larvae of Ophionotus hexactis a description can now be found in the following work: Th. Mortensen, 'Studies on the Development and Larval Forms of Echinoderms ', Copenhagen, 1921 (p. 182).

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